DECLARATION

I, Keiko Nakanishi of c/o SHIGA INTERNATIONAL PATENT OFFICE, OR Building, 23-3, Takadanobaba 3-chome, Shinjuku-ku, Tokyo, Japan, understand both English and Japanese, am the translator of the English document attached, and do hereby declare and state that the attached English document contains an accurate translation of the official certified copy of Japanese Patent Application No.10-098486 and that all statements made herein are true to the best of my knowledge.

Declared in Tokyo, Japan

This 27th day of September, 2002

Keiko Nakanishi

PATENT OFFICE

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(Title of the Invention) Monoclonal Antibody for Human Telomerase Catalytic Subunit

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[Document Type] SPECIFICATION

[Title of the Invention] Monoclonal Antibody for Human Telomerase Catalytic subunit

[Claims]

[Claim 1] A monoclonal antibody which reacts specifically with human telomerase catalytic subunit.

[Claim 2] A monoclonal antibody according to claim 1 which reacts specifically with a partial peptide of 1 - 17 units from the N-terminal of the human telomerase catalytic subunit.

[Claim 3] A monoclonal antibody according to claim 1 which reacts specifically with a partial peptide of 642 - 661 units from the N-terminal of the human telomerase catalytic subunit.

[Claim 4] A monoclonal antibody according to claim 1 which reacts specifically with a partial peptide of 1177 - 1192 units from the N-terminal of the human telomerase catalytic subunit.

[Claim 5] A monoclonal antibody according to one of claims 1 to 4 obtained by immunizing an animal with a partial peptide of the human telomerase catalytic subunit.

[Claim 6] A monoclonal antibody according to claim 5 obtained by culturing a hybridoma having the ability to produce monoclonal antibodies against the human telomerase catalytic subunit in a medium or transplanting said hybridoma into the peritoneum of an animal to induce ascitic cancer, and generating and storing said monoclonal antibodies;

said hybridoma being obtained by fusion of an antibody producing cell obtained by immunizing an animal with a partial peptide of the human telomerase catalytic subunit and a bone marrow tumor cell of an animal using normal methods.

[Claim 7] A monoclonal antibody according to one of claims 5 or 6 obtained by immunizing an animal with a partial peptide of 1 - 17 units from the N-terminal of the human telomerase catalytic subunit.

[Claim 8] A monoclonal antibody according to one of claims 5 or 6 obtained by immunizing an animal with a partial peptide of 642 - 661 units from the N-terminal of the human telomerase catalytic subunit.

[Claim 9] A monoclonal antibody according to one of claims 5 or 6 obtained by immunizing an animal with a partial peptide of 1177 - 1192 units from the N-terminal of the human telomerase catalytic subunit.

[Claim 10] KM2311 which is a mouse monoclonal antibody belonging to the IgG1 sub-class.

[Claim 11] A method for detecting specifically human telomerase catalytic subunit within cells by means of immune reaction using monoclonal antibodies which react specifically with the human telomerase catalytic subunit.

[Claim 12] A method for detecting specifically human telomerase catalytic subunit within cells according to claim 11 characterized in that said immune reaction is Western blotting.

[Claim 13] A method for detecting specifically human telomerase catalytic subunit within cells according to claim 11 characterized in that said immune reaction is immunocyte staining.

[Claim 14] A method for detecting specifically human telomerase catalytic subunit within cells according to claim 11 characterized in that said immune reaction is dot blotting.

[Claim 15] A gene which codes for a monoclonal antibody according to any one of claims 1 to 10, a partial fragment thereof or a derivative thereof.

[Detailed Description of the Invention]

[0001]

[Technical Field of the Invention]

The present invention relates to a monoclonal antibody which reacts specifically to human telomerase catalytic subunit (hereinafter referred to as hTERT). In addition, the present invention relates to a kit which uses this monoclonal antibody for the diagnosis of diseases in which telomerase is involved, and to the gene which codes for this monoclonal antibody.

[0002]

[Prior Art]

The ends of the chromosomes of eukaryotic cells like those of animal cells are called telomeres, and they have a higher-order structure comprising characteristic repetitive DNA sequences and proteins which are bonded thereto. The telomere structure is considered to have an important role in the stabilization of chromosomes. In cells in which the telomere has become shortened, deletions of chromosome and fusion of the ends of chromosomes with each other are frequently observed. In replication of straight chain DNA, since the RNA primer of the 5' terminal end cannot be replaced by DNA, the chromosome becomes shorter by the primer portion at each replication. Actually, when human somatic cells are cultured and successively passaged, the telomere becomes shortened and the cells finally die due to instability of the chromosomes as mentioned above. In this way, the length of the telomere is known as one factor which regulates the limited division potential which cells have and is known to be closely related to the aging of cells, the immortalization of cells, and the like.

[0003]

In contrast, the telomeres of protozoa and yeasts which are single celled do not shorten even when they are repeatedly propagated. In these organisms, an RNA dependent DNA polymerase, called telomerase, which lengthens one chain of the telomere repeating sequence, is active, and fixedly maintains the length of the telomere which is shortened along with division. Conventionally, measurement of the telomerase enzyme activity has been difficult

because of its low sensitivity, but a method (Telomeric Repeat Amplification Protocol; TRAP Method) has been developed which amplifies the reaction products of telomerase by PCR (Kim, N.W., Et al., Science, 266, 2011, 1995), and it is possible to measure the telomerase activity of various cells and tissues of higher animals including humans.

[0004]

Cells derived from human cancer are different from normal cells in that they can proliferate without limit *in vitro*, and the telomeres do not shorten even with repeated cell division. The results of an investigation of telomerase activity in various human tissues using the above-mentioned TRAP method indicate that telomerase activity, which is not detected in almost all normal human tissues with the exception of reproductive cells and some bone marrow cells, was characteristically detected in cancer tissues (Shay, J.W., et al., Eur. J. Cancer, 33, 787, 1997). These results indicate that the acquisition of a mechanism which avoids shortening of telomeres via telomerase is important in the development of human cancer. Since telomerase is characteristically expressed by cancer cells and concerned with their unlimited proliferation activity, it is expected that a pharmaceutical which blocks this activity will be a highly selective anti-cancer pharmaceutical.

[0005]

It is presumed that telomerase is a complex comprising a plurality of units, and the genes for the two structural units, namely, hTR (the human telomerase RNA), which is the RNA molecule which forms the template for extending a strand of the telomere DNA, and hTERT (Human Telomerase Reverse Transcriptase), which is the enzyme subunit which catalyzes the polymerase reaction, have been cloned (Feng, J. et al., Science 269, 1236, 1995; Nakamura, T.M., et al., Science 277, 955, 1997). The relationship between the expression of these units and telomerase activity has been analyzed and it has been reported that the telomerase activity characteristically detected in human cancer tissue is correlated with the

expression of the hTERT protein, i.e., the expression of telomerase activity in cancer is regulated by the hTERT protein (Nakamura, T. M., et al., Science 277, 955, 1997; Nakayama, J., et al., Nature Genetics 18, 65, 1998). In addition, it has been shown that, by the introduction of the hTERT gene into normal human cells having dividing life, the life of those cells was extended and it has become clear that hTERT functions as a molecule which regulates cell aging and the immortalization of cells (Bodnar, A.G., et al., Science 279, 349, 1998). In this way, it is expected that analysis of the function of the hTERT protein will provide important information in development of pharmaceuticals not just for cancer but for disease associated with aging.

[0006]

As a means for investigating the expression and function of specific proteins in cells and tissues, antibodies having high affinity and specificity to antigen are extremely important in the functional analysis of proteins. As antibodies for the hTERT protein, polyclonal antibodies produced using rabbits are known, and it has been reported that it is possible to detect telomerase activity in a sample obtained by immune precipitation of an extract of human cancer cells (HeLa S3) (Harrington, L., et al., Genes & Dev. 11. 3109, 1997). However, because the amount of hTERT protein expressed is extremely small, it is not possible to detect the hTERT protein expressed within the human cancer cells (HeLe S3) with the Western method using the reported polyclonal antibodies.

[0007]

[Problem to be Solved by the Invention]

An object of the present invention is providing superior monoclonal antibodies having high reactivity for the purpose of detecting the hTERT protein within cells.

[0008]

[Means for Solving the Problem]

The present invention provides a monoclonal antibody which can specifically and efficiently recognize the hTERT protein which is the catalytic subunit of telomerase and provides a kit which uses this monoclonal antibody for diagnosis of various diseases in which telomerase is involved such as cancer.

[0009]

[Embodiments of the Invention]

It is sufficient for the monoclonal antibody of the present invention to be one which reacts specifically with the hTERT which is the catalytic subunit of telomerase, but a monoclonal antibody which can be confirmed by means of the manufacturing method explained below is preferable. In more detail, it is possible to obtain an anti-hTERT monoclonal antibody by preparing hTERT, which is the catalytic subunit of telomerase, as an antigen, inducing antibody-producing cells having antigenic specificity from animals immunized with the antigen, fusing them with bone marrow tumor cells to produce hydbridomas, culturing the hybridoma or inducing ascitic cancer in an animal by administering the hybridoma to the animal; separating anti-hTERT monoclonal antibody from culture medium or the ascites and then purifying the antibody.

[0010]

[Problems to be Solved by the Invention]

In the following, the manufacturing method for monoclonal antibodies which react specifically with the hTERT protein (hereinafter referred to as anti-hTERT monoclonal antibodies) of the present invention will be explained.

As the method for preparing the antigen, a method in which an expression vector containing cDNA which codes for hTERT is prepared by known methods and then introduced into Escherichia coli, yeast, insect cells, animal cells, or the like, and thereby recombinant telomerase protein is obtained; a method in which telomerase is purified from human tumor cell

culture or the like; or a method in which a synthetic peptide (also referred to as a partial peptide) having a partial sequence of hTERT is synthesized and refined can be mentioned.

[0011]

As the partial peptide, a protein partial sequence of 5 - 30 residues is selected. In order to obtain an antibody which can recognize a protein having a natural structure, it is necessary to select a partial sequence which is present on the surface of the protein from the point of view of quaternary structure. As a method for predicting partial sequences which are present on the surface of a protein from the point of view of the quaternary structure, commercially available protein sequence analysis software, such as Genetyx Mac, and the like can be mentioned. In general, hydrophobic part of the amino acid residues is in the internal region of the structure of a protein, and hydrophilic part of the amino acid residues is on the protein surface. In addition, situations in which the N-terminal and the C-terminal of the protein are present on the protein surface are numerous. However, partial peptides selected in this way do not always give rise to antigens which produce the desired antibodies.

[0012]

In partial peptides, cysteine is added to the ends for the purpose of cross-linking with the carrier protein which will be discussed below. When an internal sequence of the protein is selected, as necessary, the N-terminal peptides are acetylized and the C-terminal peptides are amidated.

Partial peptides can be synthesized by means of general liquid phase peptide synthesis methods, solid phase peptide synthesis methods, methods which are appropriate combinations of these methods, or methods which are based on them (refer to The Peptides, Analysis, Synthesis, Biology, Vol. 1, edited by Erhard Gross and Johannes Meinhofer, Academic Press, 1979, Vol. 2, 1980, Vol. 3, 1981; Foundations and Experiments in Peptide Synthesis, Nobuo Izumiya et al, Marubeni, 1985; Development of Pharmaceuticals, New Series, Volume 14,

Peptide Synthesis, compiled by Haruaki Yajima, Hirokawa Shoten, 1991; and International Journal of Peptide Protein Research, Vol. 35, page 161 (1990)).

[0013]

In addition, it is also possible to synthesize partial peptides using an automatic peptide synthesizer. The synthesis of peptides with a peptide synthesizer can be carried out with commercially available peptide synthesizers such as peptide synthesizer manufactured by Shimadzu Seisakujo, peptide synthesizers manufactured by Applied Biosystems, Inc., USA, (hereinafter referred to as ABI Inc.), and peptide synthesizers manufactured by Advanced ChemTech Inc., USA (hereinafter referred to as ACT Inc.), using N α – Fmoc-amino acid or N α - Bmoc-amino acid in which side chains are suitably protected or the like and respective programs for synthesis.

[0014]

The protective amino acids and carrier resins used as the starting materials can be obtained from ABI Inc., Shimadzu Seisakusho, Kokusan Chemicals (KK), Nova Biochem, Watanabe Chemicals (KK), ACT Inc., Peptide Kenkyusho (KK) or the like. In addition, the protective amino acids, protective organic acids and protective organic amines which form Compounds 1 - 3 mentioned below can be synthesized according to reported synthesis methods or based on them (refer to The Peptides, Analysis, Synthesis, Biology, Vol. 1, edited by Erhard Gross and Johannes Meinhofer, Academic Press, 1979, Vol. 2, 1980, Vol. 3, 1981; Foundations and Experiments in Peptide Synthesis, Nobuo Izumiya et al, Marubeni, 1985; Development of Pharmaceuticals, New Series, Volume 14, Peptide Synthesis, compiled by Haruaki Yajima, Hirokawa Shoten, 1991; and International Journal of Peptide Protein Research, Vol. 35, page 161 (1990)).

[0015]

As the animals used in the immunization, any animal with which it is possible to make hybridomas is suitable, for example, mice, rats, hamsters, rabbits, or the like. 3 to 20 week old mice, rats or hamsters are immunized with the antigen obtained above and antibody producing cells in the spleen, lymph nodes, or peripheral blood of the animals are collected.

Immunization is carried out by administration of a suitable adjuvant (for example, Complete Freund's Adjuvant or hydroxylated aluminum gel and pertussis vaccine, or the like.) together with the antigen, subcutaneously, intravenously, or intraperitoneally. When the antigen is a partial peptide, a conjugate with a carrier protein such as BSA (bovine serum albumin) or KLH (Keyhole Limpet hemocyanin) or the like is prepared and this is used as the immunogen.

[0016]

Administration of the antigen is carried out every 1 to 2 weeks after the first administration for 5 to 10 times. On the third to seventh day after each administration, blood sample is collected from the venous plexus of the fundus oculi (eyegrounds) or caudal vein of the immunized animal, and reactivity of the serum with the antigen is confirmed by means of enzyme-linked immunosorbent assays (Antibodies - A Laboratory Manual, Cold Spring Harbor Laboratory, 1988), and immunized animals whose serum shows sufficient antibody titer with respect to the antigen used in the immunization are offered as sources of spleen cells.

3 to 7 days after the final administration of the antigen, lymphocytes are extracted from the immunized animals based on a know method (Antibodies - A Laboratory Manual Cold Spring Harbor Laboratory, 1988; hereinafter referred to as Antibodies - A Laboratory Manual), and the lymphocytes are fused with myeloma cells.

[0017]

The myeloma cells are established cell lines obtained from mice. 8-azaguanine tolerant mice (BALB/c origin) myeloma cell lines P3-X63Ag8-U1 (P3-U1) (Current Topics in

Microbiology and Immunology 18: 1-7, 1978); P3-NS1/1-Ag41(NS-1) (European J. Immunology, 6: 511-519, 1976), SP2/O-Ag14 (Sp-2) (Nature, 276:269-270, 1978), P3-X63-Ag 8653 (653) (J. Immunology, 123: 1548-1550, 1979), P3-X63-Ag8(X63) (Nature, 256, 495-497, 1975) or the like can be used. With regard to the culturing and passaging of these established cell lines, a cell number of 2×10^7 is ensured until cell fusion time according to the known methods (Antibodies - A Laboratory Manual).

[0018]

After washing the myeloma cells and the antibody producing cells obtained above, a cell agglutinative medium, such as polyethylene glycol - 1000 (PEG-1000) is added, the cells are made to fuse, and suspended within the culture medium. In the washing of the cells, MEM medium or PBS (1.83 g of disodium hydrogenphosphate, 0.21 g of potassium dihydrogenphosphate, 7.65 g of sodium chloride, and 1 liter of distilled water, pH 7.2) or the like can be used. In addition, as the medium in which the fused cells are suspended, HAT medium {a medium wherein hypoxanthine (10^{-4}M), thymidine (1.5×10^{-5}M) and aminopterin (4×10^{-7}M) are added to normal medium [a medium wherein glutamin (1.5 mM), 2-mercaptoethanol (5×10^{-5}M), gentamicin (10 μg/ml) and fetal calf serum (CFS) (10% manufactured by CSL Co.) are added to RPMI-1640 medium]}can be used in such a way that only the desired fused cells are obtained.

[0019]

After culturing, a portion of the culture supernatant is taken, and a sample is selected which reacts with the antigenic protein and which does not react with non-antigenic protein by means of enzyme-linked immunosorbent assays. Next, cloning is carried out by limiting dilution analysis, and those determined to have a stable and high antibody value by enzyme-

linked immunosorbent assay are selected as monoclonal antibody producing hybridoma cell lines.

[0020]

Enzyme-linked Immunosorbent Assays

Antigenic protein or cells that express the antigenic protein are coated on a plate and reacted with, as the first antibody, the refined antibodies obtained by the above-described method or hybridoma culture supernatant. These are then reacted with, as the second antibody, immunoglobulin antibodies (labeled with biotin, enzyme, chemiluminescent substances, radioactive compounds, or the like) for the animal tumors of the first antibodies. Then, a reaction in accordance with the labeled substance is carried out and those showing specific reactivity to the antigen are selected as hybridoma for producing monoclonal antibodies.

As the antigen, purified recombinant hTERT protein, purified hTERT protein obtained from cultured tumor cells or the like, or partial peptides of hTERT can be used. When the antigen is a partial peptide, a conjugate with a carrier protein, such as BSA (bovine serum albumin) or KLH (Keyhole Limpet hemocyanin), is prepared and this is used.

[0021]

These are coated onto plates by putting 10 - 100 μl, at 1 - 50 μg/ml, into each well of a 96 well EIA plate and allowing them to stand overnight at 4°C. Next, 100 - 200 μl of PBS solution containing 1% BSA (BSA-PBS) is put into each well and allowed to stand for 1 - 2 hours at room temperature or 1 - 2 nights at 4°C, and the residues bonded to the protein remaining on the plate are blocked (blocking). After this, the BSA-PBS is discarded, then, after washing well with PBS, as the first antibody, 20 - 100 μl of 1 - 10 μg/ml refined antibody, anti-hTERT monoclonal antibody hybridoma culture supernatant or serum of the animal to be immunized are added to each well, and allowed to stand at room temperature for 2 - 3 hours, or

overnight at 4°C. Then after washing well with PBS or PBS - 0.05% Tween, as the second antibody, 50 - 100 μl of 1 - 50 μg/ml of anti-immunoglobulin antibody labeled with biotin, enzyme, a chemiluminescent substance or a radioactive compound is added to each well, and allowed to react for 1 - 2 hours at room temperature. After washing well with PBS - Tween, a reaction is carried out in accordance with the labeling substance of the second antibody.

[0022]

Monoclonal antibodies can be prepared from the culture solution obtained by culturing hybridoma cells or by peritoneally administering monoclonal antibody producing hybridoma cells to 8 to 10 week-old mice or nude mice which have been pristane treated (intraperitoneally administering 0.5 ml of 2,6,10 4-tetramethylpentadecane (Pristane) and then rearing for two weeks), and then isolation and purification from the ascites in which cancer has been induced.

[0023]

For the isolation and purification methods for monoclonal antibodies, the following methods can be used singly or in combination: centrifugation, salting out with 40 - 50% saturated ammonium sulfate, caprylic acid precipitation, chromatography using a DEAE-sepharose column, a negative ion exchange column, a protein A or G -column, a gel filtration column, or the like. By the above methods, the IgG or IgM fractions are collected and purified monoclonal antibody is obtained.

[0024]

Determination of the subclass of the purified monoclonal antibody can be carried out using a monoclonal antibody typing kit or the like. The amount of protein can be calculated using the Lowry method or by calculation based on the optical density at 280 nm.

[0025]

In addition, the present invention relates to a method for immunologically detecting hTERT protein or cells expressing hTERT protein on the cell surface, and to a method of

immunologically detecting and assaying soluble hTERT protein, using the monoclonal antibodies of the present invention.

As the method of immunologically detecting hTERT protein and cells expressing hTERT protein on the cell surface and the method of immunologically detecting and assaying soluble hTERT protein using the monoclonal antibodies of the present invention, immunohisto staining methods, immunocyte staining methods, the Western blotting method, the dot blotting method, the above-mentioned enzyme-linked immunosorbent assay method, the sandwich ELISA method (Monoclonal Antibody Experiment Manual (Kodansha Scientific, 1987), Biochemical Experiments Lecture Series 5, Immuno-Biochemistry Research Methods (Tokyo Kagaku Dojin, 1986)) and the like can be mentioned.

[0026]

In addition, the present invention relates to diagnosis methods for diseases in which telomerase is involved, characterized by the use of the monoclonal antibodies of the present invention.

In the following, specific examples will be given.

[0027]

Western Blotting

Detection by means of Western blotting for hTERT protein using anti-hTERT monoclonal antibody can be carried out according to the following.

As the antigen, recombinant hTERT protein expressed by E. coli (1 - 10 μg/lane) can be used or a lysed cell solution (5×10^6 - 5×10^7 cells/ml) prepared from cells taken from a patient by biopsy or culturing cells of various human tumors (10 - 50 μg/lane) can be used. After fractionation by SDS-polyacrylamide electrophoresis (Antibodies - A Laboratory Manual, Cold Spring Harbor Laboratory, 1988), blotting to a PVDF sheet or nitrocellulose

sheet is carried out. After blocking with BSA-PBS, a reaction with 1 - 10 μg/ml of culture supernatant of anti-hTERT monoclonal antibodies or purified antibodies is carried out at room temperature for 2 hours or at 4°C over night. After washing well with PBS or PBS - Tween, as the second antibody, a reaction with 1 - 50 μg/ml of anti-immunoglobulin antibody labeled with biotin, enzyme, chemiluminescent substances, or radioactive compounds is carried out for 1 - 2 hours at room temperature. After washing well, a reaction in accordance with the labeling substance of the second antibody is carried out, and reaction of anti-hTERT monoclonal antibodies with protein of the molecular weight anticipated from the amino acid sequence of hTERT and that there is no reaction with antigen which does not contain hTERT is confirmed.

Western blotting using the anti-hTERT monoclonal antibody of the present invention can detect hTERT protein with high sensitivity, and is useful in the diagnosis of diseases in which telomerase is involved. The diagnosis kit provided with reagents for carrying out the Western blotting using the above-mentioned anti-hTERT monoclonal antibody makes it possible for highly reliable and convenient diagnosis with regard to diseases in which telomerase is involved.

[0028]

Dot Blotting

Dot blotting for hTERT protein using anti-hTERT monoclonal antibodies can be carried out by means of the following method.

A lysed cell solution prepared from cells taken from a patient by biopsy or culturing cells of various human tumors is dotted onto a nitrocellulose sheet at 1 - 10 μl/spot. After blocking with BSA-PBS, it is reacted with 1 - 10 μg/ml of culture supernatant of anti-hTERT monoclonal antibodies or purified antibodies at room temperature for 2 hours or at 4°C over night. After washing well with PBS or PBS - Tween, as the second antibody, a reaction with 1

- 50 μg/ml of anti-immunoglobulin antibody labeled with biotin, enzyme, chemiluminescent substances, or radioactive compounds is carried out for 1 - 2 hours at room temperature. After washing well, a reaction in accordance with the labeling substance of the second antibody is carried out.

Dot blotting using the anti-hTERT monoclonal antibody of the present invention can detect hTERT protein with high sensitivity, and is useful in the diagnosis of diseases in which telomerase is involved.

A diagnosis kit provided with reagents for carrying out the dot blotting using the above-mentioned anti-hTERT monoclonal antibody makes it possible for highly reliable and convenient diagnosis with regard to diseases in which telomerase is involved.

[0029]

Immunocyte staining

Detection by means of immunocyte staining of hTERT protein using anti-hTERT monoclonal antibodies can be carried out by means of the following method.

Free-floating cells of various human tumor cells were washed in PBS, and attached cells were washed with PBS after being made to float by washing in trypsin, EDTA or the like. In addition, the lumps of cells collected from patients by means of biopsy or the like are treated with collagenase or the like and then washed with PBS. These cells are treated with TRITON X, methanol or the like in order to improve passability for antibodies. After blocking using normal human serum or the like, 1×10^5 - 1×10^6 cells are added per tube, and reacted with 1 - 10 μg/ml of purified antibodies or culture supernatant of anti-hTERT monoclonal antibodies at room temperature for 30 minutes. Then after washing, 100 - 500 μl of 1 - 50 μg/ml of anti-immunoglobulin antibody labeled with fluorescent dye were added to each tube and

reacted at room temperature for 30 minutes. After washing well, they are mounted with glycerin and observed using a fluorescence microscope or analyzed using a cell analyzer.

Immunocyte staining using the anti-hTERT monoclonal antibody of the present invention can detect hTERT protein with high sensitivity, and is useful in the diagnosis of diseases in which telomerase is involved.

A diagnosis kit provided with reagents for carrying out immunocyte staining using the above-mentioned anti-hTERT monoclonal antibody makes it possible for highly reliable and convenient diagnosis with regard to diseases in which telomerase is involved.

[0030]

As specific examples of the anti-hTERT monoclonal antibodies selected by methods like those mentioned above, monoclonal antibody KM2311 which are produced by means of the hybridoma cell line KM2311 can be mentioned. Hybridoma cells KM2311 were deposited in accordance with the Budapest Treaty at the National Institute of Bioscience and Human-Technology as Industrial Research Organism 6306 (FERM BP-6306) on March 24, 1998.

[0031]

[Examples]

(1) Preparation of Antigen

The hTERT protein sequence was analyzed using Genetyx Mac, and from the highly hydrophilic sections, the N-terminal and the C-terminal, a partial peptide of 1 - 17 from the N-terminal of the human telomerase catalytic subunit (Compound 1, SEQ ID NO: 1), a partial peptide of 642 - 661 from the N-terminal of the human telomerase catalytic subunit (Compound 2, SEQ ID NO: 2), and a partial peptide of 1177 - 1192 from the N-terminal of the human telomerase catalytic subunit (Compound 3, SEQ ID NO: 3) were selected as partial sequences considered to be suitable as antigens.

[0032]

Codes

The code for the amino acids and the protective groups thereof which are used in the present invention are in accordance with the recommendations of the IUPAC - IUB Joint Commission on Biochemical Nomenclature [European Journal of Biochemistry, Vol. 138, Page 9, (1984)].

[0033]

The following codes represent the following amino acids except where specifically indicated otherwise.

Ala: L-alanine

Arg: L-arginine

Asn: L-asparagin

Asp: L-aspartic acid

Asx: L-aspartic acid or L-asparagin

Cys: L-cysteine

Glu: L-glutamic acid

Glx: L-glutamic acid or L-glutamine

Gly: L-glycine

Ile: L-isoleucine

Leu: L-leucine

Lys: L-lysine

Met: L-methionine

Phe: L-phenylalanine

Pro: L-proline

Ser: L-serine

Thr: L-threonine

Val: L-valine

[0034]

The following codes represent the side chain protective amino acids and protective groups of the following corresponding amino acids.

Fmoc: 9-fluororenylmethyloxycarbonyl

t-Bu: t-butyl

Trt: trityl

Pmc: 2,2,5,7,8 - pentamethylchroman - 6 - sulfonyl

Boc: t-butyloxycarbonyl

Fmoc-Thr(t-Bu)-OH: N α - 9 - fluororenylmethyloxycarbonyl - O - t - butyl - L - threonine

Fmoc-Ser(t-Bu)-OH: N α - 9 - fluororenylmethyloxycarbonyl - O - t - butyl - L - serine

Fmoc-Lys(Boc)-OH: N α - 9 - fluororenylmethyloxycarbonyl - N ε - t -butyl - oxycarbonyl - L - lysine

Fmoc - Asn(Trt) - OH: N α - 9 - fluororenylmethyloxycarbonyl - N γ - trityl - L - asparagine

Fmoc - Asp(O-t-Bu)-OH: N α - 9 - fluororenylmethyloxycarbonyl - L-aspartic acid - β - t-butylester

Fmoc-Glu(O-t-Bu)-OH: N α - 9 - fluororenylmethyloxycarbonyl - L - glutamic acid - γ - t - butylester

Fmoc-Arg(Pmc)-OH: N α - 9 - fluororenylmethyloxycarbonyl - Ng - 2,2,5,7,8 - pentamethylchroman - 6 - sulfonyl - L - arginine

Fmoc-Cys-(Trt)-OH: N α - 9 - fluororenylmethyloxycarbonyl - S - trityl - L - cysteine

[0035]

The following codes represent the following corresponding reaction solvents, reaction systems and the like.

HBTU: 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyleuronium/hexsafluorophosphate

HOBt: N-hydroxybenzotriazol

DMF: N,N-dimethylformamide

DCM: dichloromethane

TFA: trifluoroacetic acid

DIEA: diisopropylethylamine

[0036]

① Compound 1 (SEQ ID NO: 1) Synthesis of (H-Met-Pro-Arg-Ala-Pro-Arg-Ser-Arg-Ala-Val-Arg-Ser-Leu-Leu-Arg-Ser-Cys-OH)

A carrier resin bonded with side chain protective peptides was obtained using 30 mg of a carrier resin (chlorotrityl resin manufactured by AnaSpec) bonded to 14.1 μ mol of H-Cys(Trt) as the starting material, in the same way as in Example 1, by successively condensing Fmoc-Ser(t-bu)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Leu-Oh, Fmoc-Leu-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Pro-OH, Fmoc-Ala-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Pro-OH, and Fmoc-Met-OH, washing and drying. In the same way as in Example 1, cleavage of side chain protection groups and separation from the resin were carried out, 31.1 mg of crude peptide was obtained, and purified by HPLC using a reverse phase column, thereby, 4.8 mg of Compound 1 was obtained.

Mass Spectrography [FABMS]: m/z = 1956.7 ($M+H^+$)

Amino acid analysis: Ser 3.0 (3), Arg 4.7 (5), Ala 2.0 (2), Pro 2.0 (2), Val 1.0 (1), Leu 2.3 (2), met 1.0 (1), Cys 1.4 (1)

[0037]

② Compound 2 (SEQ ID No: 2) Synthesis of (Ac-Ala-Arg-Thr-Phe-Arg-Arg-Glu-Lys-Arg-Ala-Glu-Arg-Leu-Thr-Ser-Arg-Val-Lys-Ala-Cys-OH)

30 mg of carrier resin (chlorotrityl resin manufactured by AnaSpec) bonded to 14.1 μmol of H-Cys(Trt) was put into the reaction chamber of an automatic synthesizer (Shimadzu Seisakujo), 1 ml of DCM/DMF (1:1) were added, stirred for 10 minutes and the solution discharged. In addition, 1 ml of DMF was added, stirred for 1 minute and the solution discharged, then the following operations were carried out in accordance with the program for synthesis of the Shimadzu Seisakusho.

(a) Fmoc-Ala-OH (141 μmol), HBTU (141 μmol), hydrate of H0Bt1 (141 μmol) and DIEA (282 μmol) in DMF (734 μl) were stirred for 3 minutes, and the obtained solution was added to resin and the mixture was stirred for 30 minutes, and then the solution discharged.

(b) The carrier resin was washed for 1 minute with 734 μl of DMF and this was repeated five times. In this way, Fmoc-Ala-Cys(Trt) was synthesized on the carrier.

[0038]

Next the following Fmoc group protection removal process was conducted.

(c) 734 μl of 30% piperidine - DMF solution was added and the mixture stirred for four minutes, this solution was then discharged and this operation was repeated once.

(d) The carrier resin was washed for 1 minute with 500 μl of DMF, this solution was discharged, and this operation was repeated five times.

In this way, a carrier resin bonded with H-Ala-Cys(Trt) having the Fmoc group removed was obtained.

[0039]

Next, H-Lys(Boc)-Ala-Cys(Trt) was synthesized on a carrier by conducting a condensation reaction using the Fmoc-Lys(Boc)-OH using the process of (a), and by passing through the washing process of (b), and the protection removal processes of (c) and (d). Next, a carrier resin bonded to a side chain protection peptide was obtained by successively using Fmoc-Val-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Leu-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Ala-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Phe-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Ala-OH, then, after repeating (a) - (d), washing successively with methanol and butylether and drying under reduced pressure for 12 hours. To this, 1 ml of a solution mixture comprising TFA (82.5%), theoanisol (5%), water (5%), ethyl methyl sulfide (3%), 1,2-ethanedithiol (2.5%) and thiophenol (2%) was added and allowed to stand for 8 hours at room temperature, and the side chain protection units were remove while the peptide was separated from the resin. After filtering the resin, approximately 10 ml of ether were added to the obtained solution, the produced precipitate was collected by centrifugation and decantation, and thereby 36.2 mg were collected as crude peptide. After washing this crude product in 2 M acetic acid, it was purified by HPLC using a reverse phase column (CAPCELL PAK C18 30 mmI.D. X 25 mm, manufactured by Shiseido). The fraction containing Compound 1 was obtained by elution using a straight line concentration gradient method carried out with the addition of 90% acetonitrile aqueous solution containing 0.1% TFAO to a 0.1% TFA aqueous solution, and detection at 220 nm. 2.3 mg of Compound 2 was obtained by freeze drying this fraction.

Mass Spectrography [FABMS]: m/z = 2477.4 ($M+H^+$)

Amino acid analysis: Glx 2.0 (2), Ser 1.2 (1), Arg 5.4 (6), Thr 2.0 (2), Ala 3.2 (3), Val 1.0 (1), Leu 1.3 (1), Lys 2.0 (2), Phe 0.8 (1), Cys 1.5 (1)

[0040]

③ Compound 3 (SEQ ID NO: 3) Synthesis of (H-Cys-Ala-Ala-Asn-Pro-Ala-Leu-Pro-Ser-Asp-Phe-Lys-Thr-Ile-Leu-Asp-OH)

A carrier resin bonded with side chain protective peptides was obtained using 30 mg of a carrier resin (Wang resin manufactured by NovaBioche) bonded to 14.1 μ mol of Fmoc-Asp(0t-Bu) as the starting material, by after carrying out processes (c) and (d) in Example 1, successively condensing Fmoc-Leu-OH, Fmoc-Ile-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-Asp(Ot-Bu)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Pro-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Pmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ala-OH, Fmoc-Ala-OH, Fmoc-Cys(Trt)-OH, washing and drying in the same way as in Example 1. In the same way as in Example 1, cleavage of side chain protection groups and separation from the resin were carried out, 27.6 mg of crude peptide was obtained, and purified by HPLC using a reverse phase column, thereby, 10.3 mg of Compound 3 were obtained.

Mass Spectrography [FABMS]: m/z = 1675.6 ($M+H^+$)

Amino acid analysis: Glx 3.0 (3), Ser 1.1 (1), Ala 3.0 (3), Pro 2.1 (2), Leu 2.0 (2), Lys 1.0 (1), Ile 0.9 (1), Phe 1.0, Cys 1.0 (1)

[0041]

(2) Preparation of Immunogen

In order to increase immunogenicity, the hTERT partial peptide obtained in Example 1 (1) was made into a conjugate with KLH (Carbochem Co.) using the following method and used as an immunogen. That is, 10 mg/ml of KLH was prepared by dissolving in PBS, then an amount equivalent to 1/10th of the total volume of 25 mg/ml MBS (Nakaraitesku Co.) was added dropwize and reacted with stirring for 30 minutes. 2.5 mg of KLH-MB obtained by the removal of free MBS using a gel filtration column such as Sephadex G-25 equilibrated with PBS in advance was mixed with 1 mg of peptide dissolved in 0.1 M sodium phosphate buffer

(pH 7.0) and reacted while stirring for 3 hours at room temperature. After the reaction, dialysis was conducted with PBS - 0.5 M NaCl.

[0042]

(3) Immunization of Animals and Preparation of Antibody Producing Cells

100 μg of the peptide - KLH conjugate prepared in Example 1 (2) were administered together with 2 mg of aluminum gel and 1×10^9 cells of pertussis vaccine (manufactured by Chiba Prefecture Serum Laboratory) to 5 week old female mice (Balb/c), then, two weeks later, 100 μg of conjugate was administered once a week for a total of 4 times. Blood was collected from the venous plexus of the fundus oculi (eyegrounds) and the serum antibody titer was determined by enzyme-linked immunosorbent assay as shown below. Three days after the last immunization, the spleen was excised from mice who showed sufficient antibody titer.

[0043]

The spleen was cut to pieces in MEM medium (manufactured by Nissui Seisakujo), loosened using tweezers, and centrifuging (1,200 rpm, for 5 minutes), the supernatant discarded, removed red blood cells by treating with Tris - ammonium chloride buffer (pH 7.65) for 1 to 2 minutes, washed with MEM medium 3 times, and used in the cell fusion.

[0044]

(4) Enzyme-linked Immunosorbent Analysis

In the antigen for the assay, one in which the hTERT partial peptide obtained in Example 1was conjugated with thyroglobulin (hereinafter abbreviated as THY) was used. The preparation method was the same as described in Example 1 (2), except that SMCC (Sigma Co.) was used in place of MBS as the cross-linking agent. To a 96 well EIA plate (Griener Co.), 50 μl of the 10 μg/ml conjugate prepared in the above mentioned way was added to each well and allowed to stand over night at 4°C to absorb. After washing, 100 μl of 1% BSA-PBSA

was added to each well, reacted for 1 hour at room temperature, and the remaining active groups blocked. The 1% BSA-PBS was discarded, and 50 μl of antiserum of the mouse to be immunized, culture supernatant of the anti-hTERT monoclonal antibody or purified monoclonal antibodies were added to each well and allowed to react for 2 hours. After washing with tween - PBS, 50 μl of rabbit anti-mouse immunoglobulin labeled with peroxydase (Dako Co.) was added to each well and allowed to react for 1 hour at room temperature, then, after washing with tween - PBS, color was developed using ABTS matrix liquid [2.2-adinobis (3-ethylbenzothiazol-6-sulfonic acid) ammonium] and OD415 nm absorbance was measured using a plate reader (NJ 2001; manufactured by Nihon Intermed Co.)

[0045]

(5) Preparation of the Mouse Myeloma Cells

8-azaguanine resistant mouse bone marrow tumor cell line P3-U1 was cultured in normal medium, more than 2×10^7 cells were obtained at the time of cell fusion, and submitted as a parental strain in the cell fusion.

[0046]

(6) Preparation of Hybridoma

Mouse spleen cells obtained in Example 1 (3) and bone marrow tumor cells obtained in (5) were mixed so as to be 10:1, after centrifugation (1,200 rpm, for 5 minutes), the supernatant was discarded, and the precipitated cells were well loosened, then, while stirring, 0.2 - 1 ml of a liquid mixture of 2 grams of polyethylene glycol - 1000 (PEG-1,000), 2 ml of MEM medium, and 0.7 ml of dimethylsulfoxide was added per 10^8 mouse spleen cells, and then after adding 1 - 2 ml of MEM medium ever 1 - 2 minutes several times, MEM medium was added so as to give a total amount of 50 ml. After centrifugation (900 rpm, for 5 minutes), the supernatant was

discarded, cells were made loose, then the loosened cells were suspended in 100 ml of HAT medium by being drawn up into a measuring pipette and then discharged.

[0047]

100 μl of this suspension were added to each well of a 96 well culturing plate, and cultured under 5% CO_2 for 10 - 14 days at 37°C in a 5% CO_2 incubator. The supernatant of this culture was tested using the enzyme-linked immunosorbent assay described in Example 1 (4), wells which reacted with the hTERT partial peptide and did not react with the control peptide were selected, and, changing the HT medium with normal medium, cloning was repeated two times, thereby establishing the anti-hTERT monoclonal antibody producing hybridoma.

[0048]

Using Compound 1 (SEQ ID NO: 1), 2 monoclonal antibodies, KM2294 and KM 2295 were selected; using Compound 2 (SEQ ID NO: 2), 8 monoclonal antibodies, KM2277, KM2278, KM2279, KM2280, KM2281, KM2282, KM2283 and KM2284 were selected; and using Compound 3 (SEQ ID NO: 3) in the antigen, 17 monoclonal antibodies, KM2296, KM2297, KM2298, KM2299, KM2300, KM2301, KM2302, KM2303, KM2304, KM2305, KM2306, KM2307, KM2308, KM2309, KM2310, KM2311, and KM2312 were selected.

[0049]

(7) Purification of Monoclonal Antibody

Cells of the hybridoma lines obtained in Example 1 (6) were injected peritoneally into 8 week old, female, nude mice (Balb/c) treated with pristane in an amount of $5 \sim 20 \times 10^6$ cells/animal. 10 ~ 21 days later, the hybridoma produced ascitic cancer. Ascites (1 - 8 ml/animal) was collected from mice in which ascites had accumulated, and the solid portion removed by centrifugation (3,000 rpm, for 5 minutes). When the monoclonal antibodies were IgM, salting out was conducted with 50% ammonium sulfate, and after dialysis with PBS to

which 0.5 M of sodium chloride had been added, the IgM fraction was collected by passing through a column of Cellulofine GSL 2000 (Seikagaku Industries KK) (750 ml bed volume) at a rate of 15 ml/hour, to obtain purified monoclonal antibodies. When the monoclonal antibodies were IgG, purified monoclonal antibodies were obtained by purification using caprylic acid precipitation (Antibodies - A Laboratory Manual, Cold Spring Harbor Laboratory, 1988).

The subclass of the antibodies was determined by enzyme-linked immunosorbent assay using a sub-class typing kit (Table 1).

[0050]

[Table 1]

[0051]

(8) Reactivity of the hTERT partial peptide (enzyme-linked immunosorbent assay)

Reactivity of the anti-hTERT monoclonal antibody selected in Example 1 (6) with the hTERT partial peptide was investigated using the enzyme-linked immunosorbent assay shown in (4).

As shown in Figure 1, the anti-hTERT monoclonal antibodies (KM2294 and KM2295) obtained using Compound 1 (SEQ ID NO: 1) reacted specifically with Compound 1. As shown in Figure 2, the anti-hTERT monoclonal antibodies (KM2277 to KM2284) obtained using Compound 2 (SEQ ID NO: 2) reacted specifically with Compound 2. As shown in Figure 3, the monoclonal antibodies (KM2296 to KM2312) obtained using Compound 3 (SEQ ID NO: 3) in the antigen reacted specifically with Compound 3.

[0052]

(9) Western Blotting

Detection of hTERT protein within cells by Western blotting was studied using the anti-hTERT monoclonal antibodies selected in Example 1 (6).

Three types of cells, human renal transformant 293 (ATCC CRL1537), human cervical cancer cell line HeLaS3 (ATCC CCL - 2.2) and normal human lung cells MRC5 (ATCC CCL212), were used. Cells of these lines were floated in a tripsin and EDTA solution mixture (Sankou Junyaku) and washed in PBS. 1 ml of buffer for cytolysis (50 mM Tris-HCL, pH 7.2, 1% TritonX, 150 mM NaCl, 2mM $MgCl_2$, 2mM $CaCl_2$, 0.1% NaN_3, 50mM iodoacetamide, 50mM N-ethylmaleidmide, 1 mg/ml leupepcin, and 0.1 mM dithiothreitol) was added to 5×10^7 cells, and allowed to stand for 2 hours at 4°C, and then centrifuged. After fractionation of the obtained supernatant by SDS-electrophoresis with 10^5 cells per lane (Antibodies - A Laboratory Manual, Cold Spring Harbor Laboratory, 1988), blotting to a PVDF membrane was carried out. After blocking with BSA-PBS, the culture supernatant of the anti-hTERT monoclonal antibodies was allowed to react for 2 hours at room temperature. After washing well with PBS-Tween, a reaction with anti-mouse immunoglobulin antibody (Dako KK) labelled with peroxidase as the second antibody was carried out for 1 hour at room temperature. After washing well with PBS - Tween, detection was carried out using an ECL detection kit (Amersham Co.), and sensitized on X-ray film. The results are shown in Figure 4. In Figure 4, Lane 1 shows the results for the 293 cytolysis solution, Lane 2 shows the results for the HeLaS3 cytolysis solution, and Lane 3 shows the results for the MRC 5 cytolysis solution.

[0053]

As shown in Figure 4, using KM2311 (the anti-hTERT monoclonal antibodies obtained by means of Compound 3), bands were detected in the cytolysis solution of 293 cells and HeLaS3 cells in the vicinity of 130 KDa which corresponds to the molecular weight of hTERT. In addition, in the cytolysis solution of the MRC5 cells, which are normal cells, a specific band was not detected. With regard to monoclonal antibodies other than KM2311, none reacted specifically in the vicinity of 130 KDa band.

The above results show that KM2311 is able to detect hTERT protein within cells by Western blotting and that it can be used in the diagnosis of diseases, such as cancer, in which telomerase is involved.

[0054]

(10) Dot Blotting

Detection of hTERT protein within cells by dot blotting was studied using anti-hTERT monoclonal antibodies. As the anti-hTERT monoclonal antibodies, the culture supernatant of KM2311 detected by hTERT protein in Example 1 (7) was used.

Cytolysis solution of human renal transformant 293 cells prepared in Example 1 (7), in a concentration of 2.5 X 10^5 cells/5 μl were diluted by 2, 4, 8, 16, 32 and 64 times with cytolysis buffer, and then each of these was dotted onto a nitrocellulose membrane in an amount of 5 μ l per spot. After drying, and after blocking with BSA-PBS, they were reacted with KM2311 culture supernatant for 2 hours at room temperature at undiluted solution. After washing well with PBS-Tween, a reaction with anti-mouse immunoglobulin antibodies labeled with peroxidase as the second antibody was carried out for 1 hour at room temperature. After washing well with PBS-Tween, detection was carried out using an ECL-detection kit (Amersham CO.) and sensitized on an X-ray film. The results are shown in Figure 5. In Figure 5, the upper column is the result of KM511 and the lower column is the result of KM2311. In Figure 5, the results of the reaction with the nitrocellulose membrane on which the cytolysis solution of human renal transformant 293 was spotted, from the left, as undiluted solution, 2, 4, 8, 16, 32, and 64 times dilutions, are shown.

As shown in Figure 5, KM2311 is able to detect hTERT protein within cells by dot blotting and that it can be used in the diagnosis of diseases, such as cancer, in which telomerase is involved.

[0055]

(11) Immunocyte staining

Detection of hTERT protein within cells by means of immunocyte staining was studied using the anti-hTERT monoclonal antibodies selected in Example 1 (6).

A total of three cell lines, human renal transformant 293, human cervical cancer cell line HeLaS3 and normal human lung cells WI-38 (ATCC CCL75), were used. Cells of these lines were floated in a tripsin and EDTA solution mixture and washed in PBS. Then, in order to increase penetrability of the cell membrane by antibodies, they were treated for 10 minutes at 4°C in 100% methanol (chilled on ice). After washing with PBS, blocking was carried out for 30 minutes at room temperature with 10 μg/ml of human immunoglobulin (Cappel Co.). After adding 1×10^5 cells per tube, centrifugation was conducted and the supernatant removed, and culture supernatant of the anti-hTERT monoclonal antibody added and allowed to react for 30 minutes at room temperature. After washing in PBS, 100 μl of anti-mouse immunoglobulin antibody (specific for mouse immunoglobulin, Wako Junyaku) was added per tube, and allowed to reacted with shielding for 30 minutes at 4°C. After washing well with PBS, analysis using a cell analyzer (Coulter Co; EPICS XL system II) was carried out.

Figure 6 shows charts of the cell analysis, and the results with regard to each of the 293 cells (left column), HeLaS3 cells (center column) and WI38 cells (right column) were shown in case of the addition of KM2311 (upper graph), KM511 (middle column), and BSA (lower column).

By the peak shift of Figure 6, the reactivity of KM2311 with 293 cells and HeLaS3 cells is recognized. In addition, reactivity with WI38, which are normal cells, is not recognized. Monoclonal antibodies other than KM2311 did not show specific reactivity to cancer cell lines.

Consequently, KM2311 is able to detect hTERT protein within cells by means of immunocyte staining and shows that it can be used in the diagnosis of various diseases, such as cancer, in which telomerase is involved.

[0056]

[Effects of the Invention]

According to the present invention, anti-hTERT monoclonal antibodies are provided which react specifically with hTERT and which detect specifically hTERT protein by Western blotting, immunocyte staining and dot blotting.

By these methods, anti-hTERT monoclonal antibodies which detect hTERT protein specifically and diagnosis kits which use them make highly reliable and highly sensitive detection possible in the diagnosis of diseases in which telomerase is involved.

[0057]

[Sequence Listing]

SEQ ID NO: 1

SEQUENCE LENGTH: 17

SEQUENCE TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE

Met Pro Arg Ala Pro Arg Ser Arg Ala Val Arg Ser Leu Leu Arg Ser Cys

1 5 10 15

[0058]

SEQ ID NO: 2

SEQUENCE LENGTH: 20

SEQUENCE TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

FEATURE

Feature Key: modified-site

Location: 1

Other Information: Xaa represents N-acetyl-L-alanine

SEQUENCE

Xaa Arg Thr Phe Arg Arg Glu Lys Arg Ala Glu Arg Leu Thr Ser Arg Val Lys Ala Cys

1 5 10 15 20

[0059]

SEQ ID NO: 3

SEQUENCE LENGTH: 16

SEQUENCE TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE

Cys Ala Ala Asn Pro Ala Leu Pro Ser Asp Phe Lys Thr Ile Leu Asp

1 5 10 15

[Brief Description of the Drawings]

[Figure 1] is a graph showing the results of reactivity for Compounds 1 and 2 of the monoclonal antibodies of the present invention obtained using Compound 1 as an antigen, by enzyme-linked immunosorbent assay.

[Figure 2] is a graph showing the results of reactivity for Compounds 2 and 3 of the monoclonal antibodies of the present invention obtained using Compound 2 as an antigen, by enzyme-linked immunosorbent assay.

[Figure 3] is a graph showing the results of reactivity for Compounds 1 and 3 of the monoclonal antibodies of the present invention obtained using Compound 3 as an antigen, by enzyme-linked immunosorbent assay.

[Figure 4] is a photograph showing the results of the detection of hTERT protein present within cells by Western blotting using the monoclonal antibodies of the present invention.

[Figure 5] is a photograph showing the results of the detection of hTERT protein present within cells by dot blotting using the monoclonal antibodies of the present invention.

[Figure 6] is a chart showing the results of the detection of hTERT protein present within cells by immunocyte staining using the monoclonal antibodies of the present invention.